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TITLE TESTING ANTI-hPTK6-IgG-FUSION - SERAProj No 9211
Book No. _____

Exhibit L, pg. 1 of 31

From Page No. _____

Aim

Two rabbits have been immunised with the
hptk6-IgG fusion protein purified by W. Baron.

These sera will contain Ab's against both the
human hptk6 and the IgG (Fc) domain.

- Need to test these sera

Ran 20µg of a) hptk6-IgG fusion protein
b) hptk5-IgG fusion protein

on 8% SDS - polyacrylamide gels

; Blotted to NC filters

Ponceau stained filters after transfer &
prepared strips for testing

- Photocopies of filters & attached over.

To Page No. _____

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Date _____

Recorded by _____

30th Nov 97

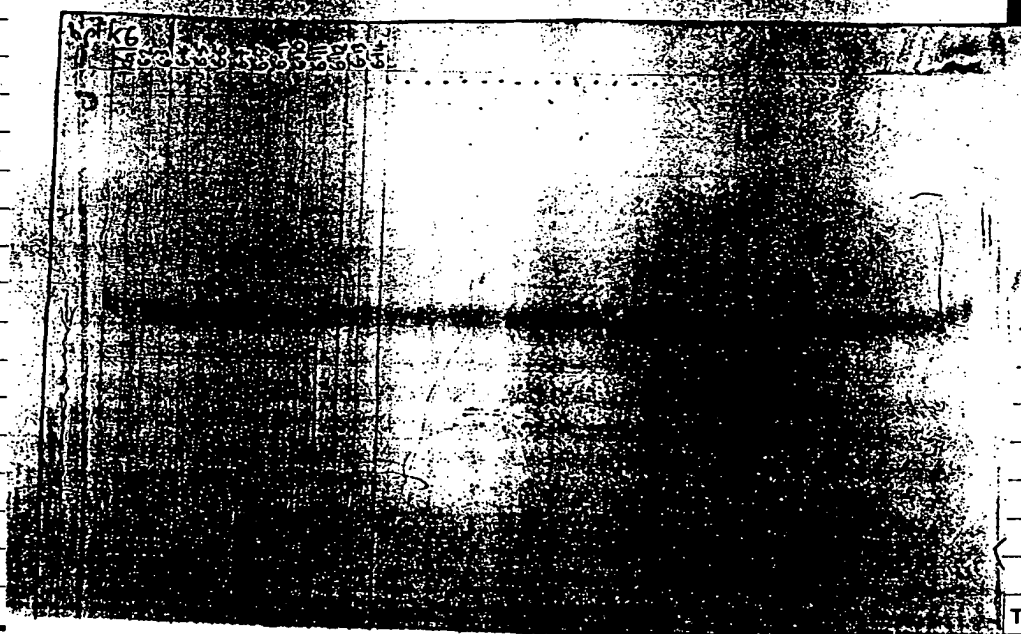
Project No. _____
Book No. 19211 TITLE TESTING & HPTK6 - SERA

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30 Nov 93

TITLE

TESTING OF HPTK-6 SERA.

Project No.

Book No. 19211

Exhibit L, pg. 3 of 31

From Page No. _____

Two batches of test serum obtained together
with prebleed controls

Rabbit #	PRE-BLEED DATE	BLEED AFTER APR SECOND BOOST
18243 - 71A	8/11/93	9/30/93
18243 - 71B	8/11/93	9/30/93

Used strips as follows

Strip #	SERUM	DIL'N	VOLUME
1	71A (Prebleed)	1/100	100 μ
2	71A	1/100	100 μ
3	71A	1/1000	10 μ
4	NONE - SECONDARY ALONE.		
5	71B (Prebleed)	1/100	100 μ
6	71B	1/100	100 μ
7	71B	1/500	20 μ
8	71B	1/1000	10 μ

Developed using 2° anti-rabbit - Phosphatase (Boehringer) with BCIP + NBT.

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30th Nov. 93

Project #

Book No. 19211

TITLE

TESTING 2 HPTK6 SERA

Exhibit L, pg. 4 of 31

From Page No. _____

0.69

0.70

0.71

0.72

0.73

0.74

0.75

0.76

0.77

0.78

0.79

0.80

0.81

0.82

0.83

0.84

FRIDAY 5th Nov 93

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30th Nov 93

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TITLE TESTING X HPTK6 SERA

Proj No. _____
Book No. 9211

Exhibit L, pg. 5 of 31

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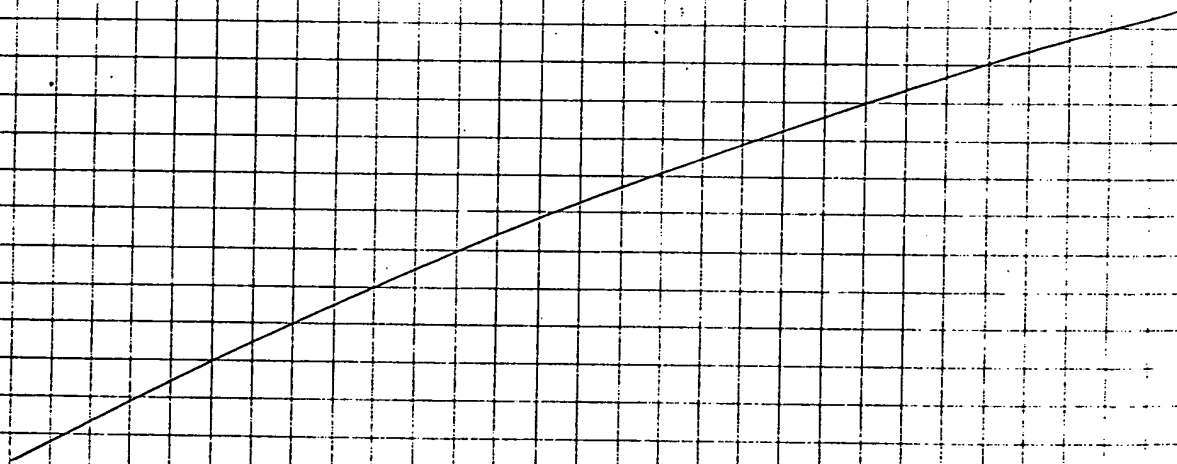
RESULTS & CONCLUSIONS

There appears to be a good differential reaction with the 71-B serum when used at especially 1:1000 dilution.

- There is a much stronger reaction against hptk6 than hptk5 on strip #8.

This can also be seen to a lesser extent with the lower dilutions of 71B, and perhaps with the 71A serum also.

The prebleeds and the no-primary controls are clean



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30 Nov. 93

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Project # _____
Book No. 19211 TITLE SCREENING CHO-FULL LENGTH CLONES

From Page No. _____

Aim

Use the 71-B antisera to screen the 20 clones that will screen no generated that potentially express full-length PPTK-6.

Plates of 20 CHO cell lines were used; also used untransfected CHO cells were confluent and grown in F12/DMEM -GHT.

Remove medium from cells, rinsed in PBS, then added 10mls. PBS + 5mM EDTA - left at 37°C for 20-30 mins.

Cells were transferred to 15ml falcon tubes, washed 2x with PBS & then stored frozen at -20°C.

(21st Nov 93)

- The rinsed cells were lysed by resuspension into 450µl of RIPA buffer + PMSF + Aprotinin, left on ice for 15 mins prior to spinning 2-3 mins at 2000 rpm in the microfuge.

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30th Nov 93

TITLE

SCREENING full LENGTH EXPRESSION.

Proj No. _____

Book No. 921

Exhibit L, pg. 7 of 31

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From Page No. _____

400 μ l of the supernatant was transferred to a new tube and 200 μ l of 3x Sample Buffer (+ β -ME) was added.

→ Samples were heated at 95°C for 10 mins.

80 λ aliquots loaded onto 8% SDS-PAGE gels.

GELS 1+2 ;

Mr 1 → 15

GEL 3

Mr 16, 17, 18, 19, 20, CHO [] Mr 16 → 20, CHO.

Ran slowly o/n.

(22nd Nov '93)

Blotted gels to N/C filters as normal,

Ponceau stained - photocopies shown overleaf, (Page 8)

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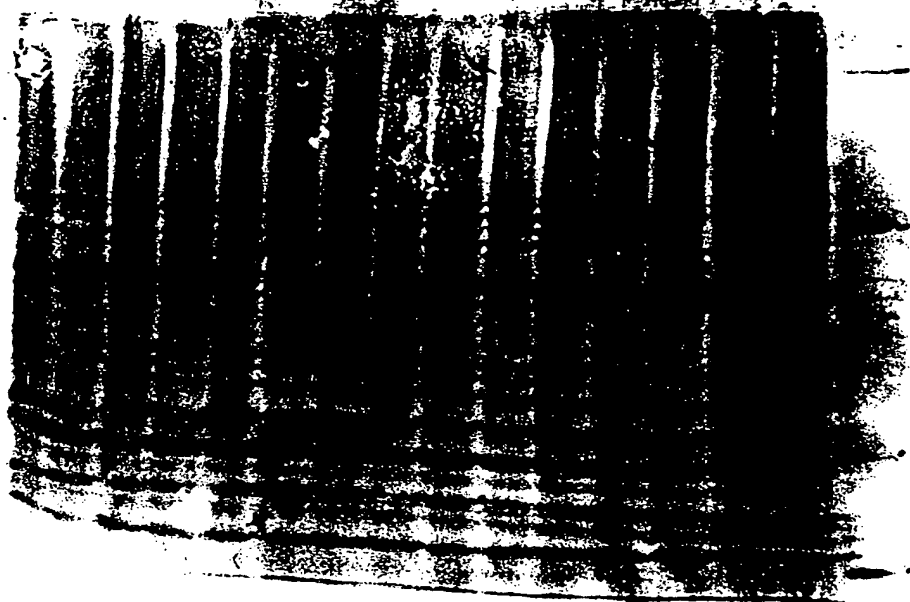
Project I.

Book No. 19211 TITLE

SCREENING Unit 1 (LAW) 2-11-93

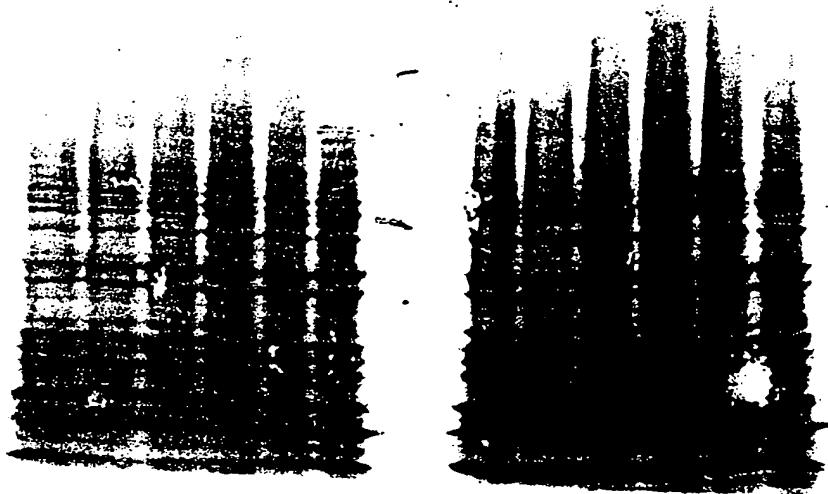
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CP. PUN

30 Nov 93

TITLE

SCREENING FULL LENGTH EXPRESSION

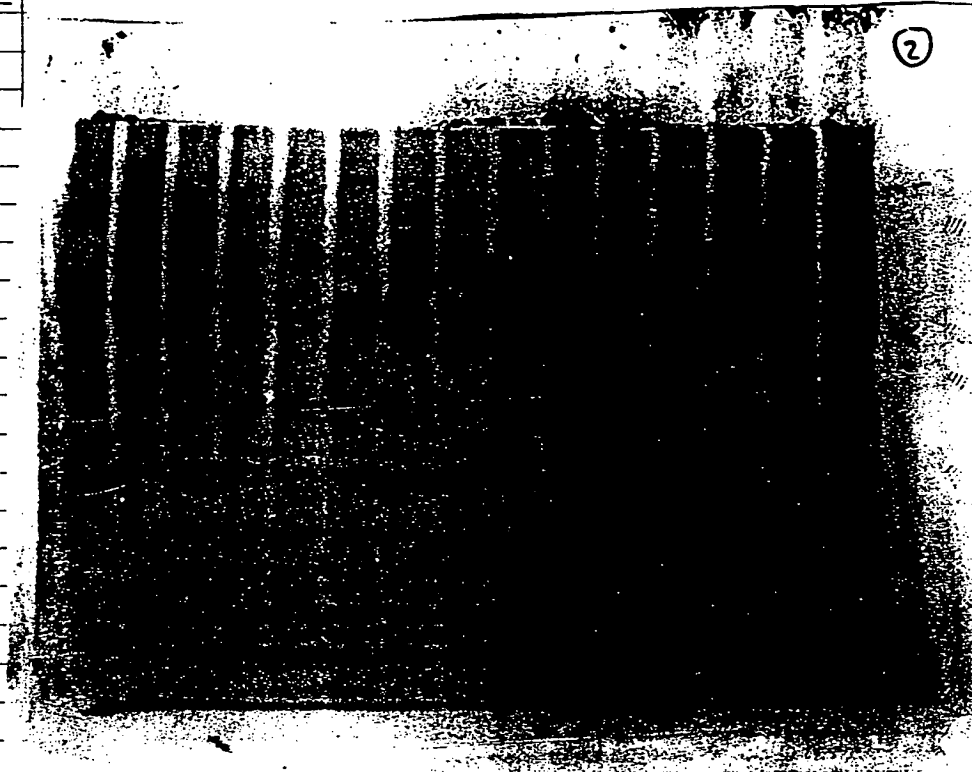
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Book No. 9211

Exhibit L, pg. 9 of 31

From Page No.



Gel 3 split with 2 experiments - gels 3+4.

GEL 1 + 3 probed with 1/1000 anti-hptk-6 F1B
as used previously ;

Gels 2 + 4 Probed with UBI monoclonal
anti - (P)-Tyr.

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30th Nov 93

From Page No. —

Developed with 2°-anti rabbit or anti-mouse (P-Tyr) - phosphate linked
& BCIP + NBT

Alkaline Phosphate Reaction:

- Wash Blot with Alk Phosph. Buffer 5 min.

- Make up Reaction Soln: 20 ml Alk Phosph Buff.
132 μ l Nitro blue tetrazolium
66 μ l BCIP

RESULTS & CONCLUSIONS

Blots are shown on Page 11 for α hptk-6
Page 12 for α - (P-Tyr).

Sample # 18 appears to have a band of the
approx. right Mr coming up - this may also be
present to a lesser extent in #19 & #20 too;
+ much lower in #4.

In addition a band of much higher Mr ^{than expected} appears to be
expressed well in #16 and to a lesser extent in
#1, #17, #18.

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J.P. [Signature]

30th Nov 93

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Book No. 9211

Exhibit, pg. 11 of 31

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30th Nov 93

ANTI - HPTK6

Proj. No. _____
Book No. 19211 TITLE _____

Exhibit L, pg. 12 of 31

From Page No. _____

(2)

15

16

4

CWP/10

ANTI - (P) - T&R

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30th Nov. 83

From Page No.

The anti-phosphotyrosine blot shows no band of the right mol. wt for hptk-6, suggesting that the receptor is not phosphorylated under these conditions - Note I did not add phosphatase inhibitors to the cells when I lysed them and this may be important!

However, there is a distinct band of approx 80 kDa coming up in most of the transfected samples cf. CHO cells alone. Moreover this band appears strongest in sample #18.

This may be related to hptk-6 expression - perhaps a target for the kinase

These results should tie in with whatever Will Baron finds out from a FACS analysis of these 20 cell lines using this criterion.

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229. PEN30th Nov. 93

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Project No. _____
Book No. 19211 TITLE TESTING FURTHER α HPK6 BLEEDS

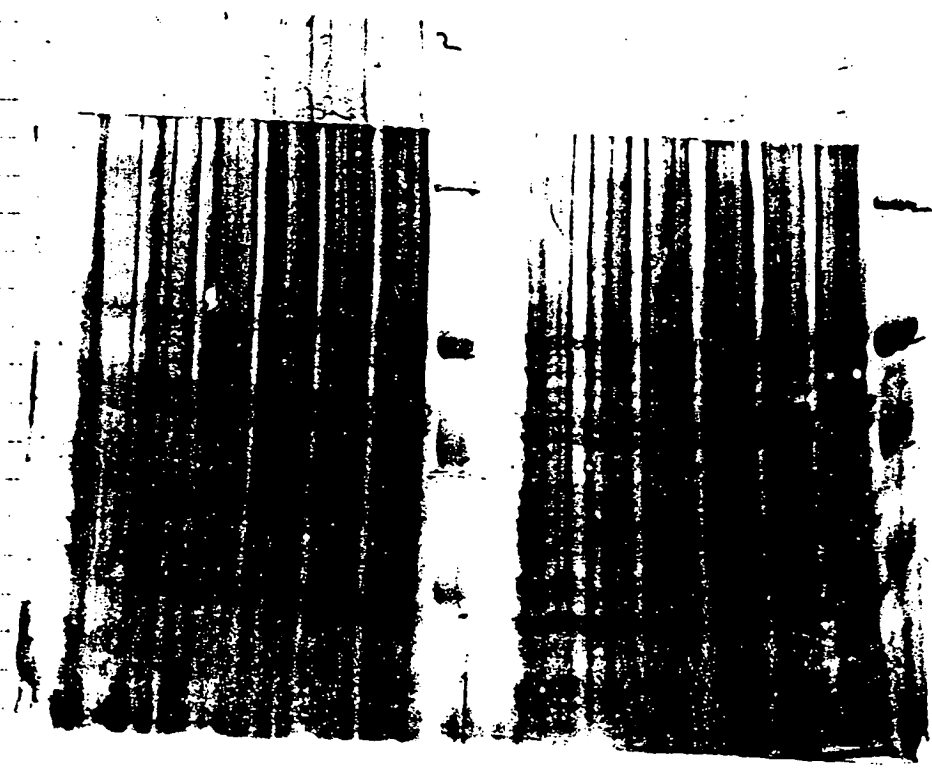
From Page No. _____

Monday 6th Dec 93.

Ran 6 μ l aliquots of samples prepared on Nov 21st 93
on 0.8% gel.

Ran gel fast (~60min)

Blotted to NK Filter ; Ponceau Stained + photocopied.



Gel overloaded
+ ran too fast!!

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Recorded by K P M W

Dec 7th 93

TITLE

TESTING FURTHER BLOODS

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Book No.

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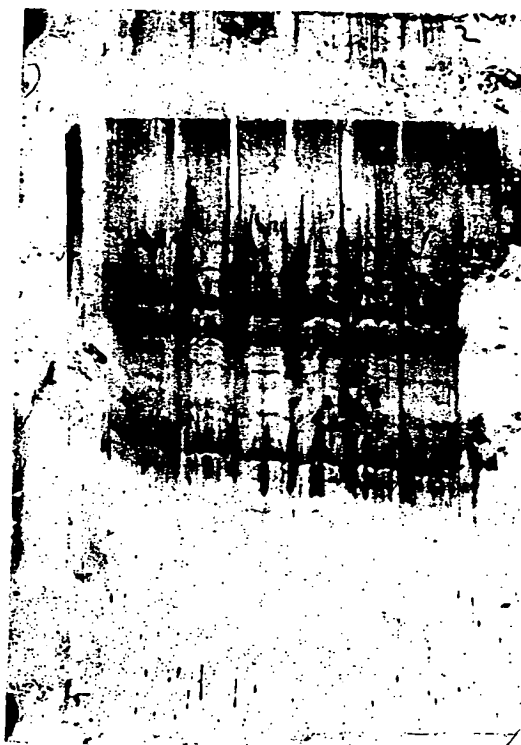
Exhibit L, pg. 15 of 31

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Tuesday 7th Dec. 93

Obtained 2 new bleeds from rabbit 71-B
 Recd from 10/28/93 & 11/18/93.

Used 1/500 dilutions of these to decorate the
 blot
 Panel (1) 10/28/93
 Panel (2) 11/18/93



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J P Blv

7th Dec 93

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RESULTS & CONCLUSIONS

Blot far too overloaded + messy to make any definite conclusions about this.

- Need to repeat using perhaps 30% aliquots of the surplus.

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7 Dec 93

TITLE TESTING HPMK6 BLEEDS AGAIN.

From Page No. —

7th Dec. 93

Prepared 2 new 8% gels.
loaded with samples as previous

i.e. Mr 16, 17, 18, 19 20 CHO, Mr 16, 17, 18, 19 20 CHO

Run gels slowly o/n.

8th Dec. 93

Transferred to N/C filters

Process stained — copies attached page 18,

Blotted with

PANEL

PROBE

1

7IB

2

7IB

3

7IB — Pre bleed.

4

No Primary Ab.

— 2 hrs RT; Wash, then Probed all with 1:1000 of

Anti-Rabbit — Alk Phosph. conjugate

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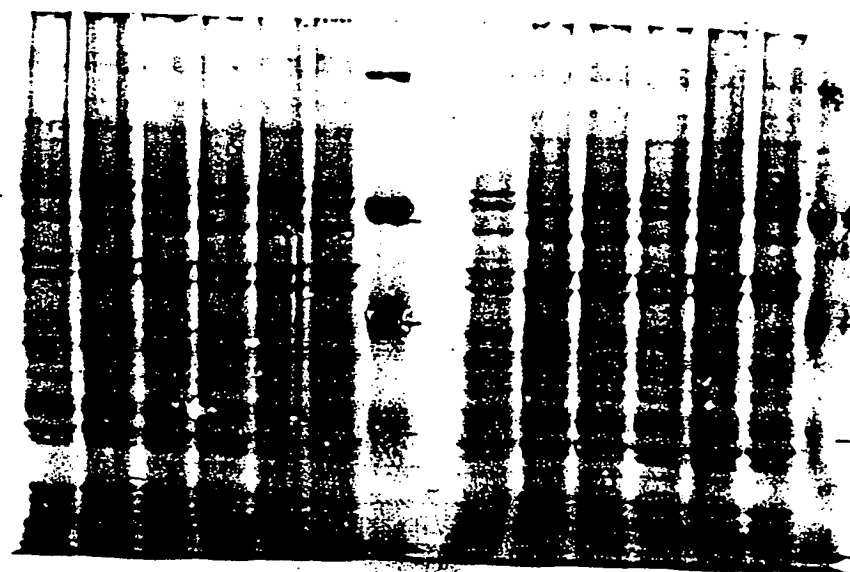
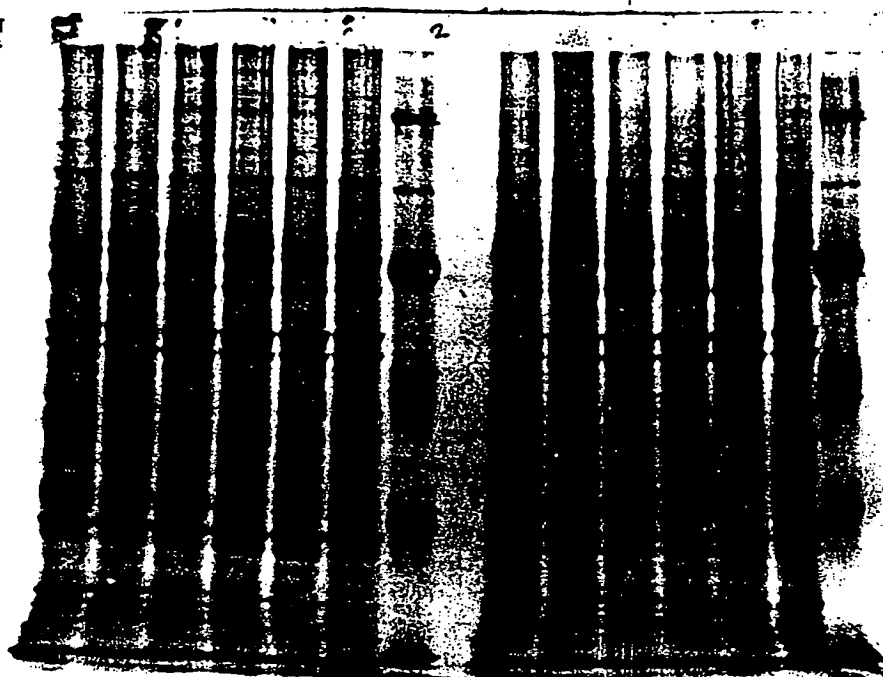
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9th Dec 93

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Dec 93

TITLE

TESTING 2 HPTK-6 BLOOD AGAIN.

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Book No.

Exhibit L, pg. 19 of 31

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Developed with BCIP / NBT as used.

;- Westerns attached Page 20

RESULTS & CONCLUSIONS

- Gels are much cleaner than before, although

I do not see any major bands that are obvious in the transfected cell lines of CHO untransfected.

There are a couple of minor bands at about the approx. right Mr

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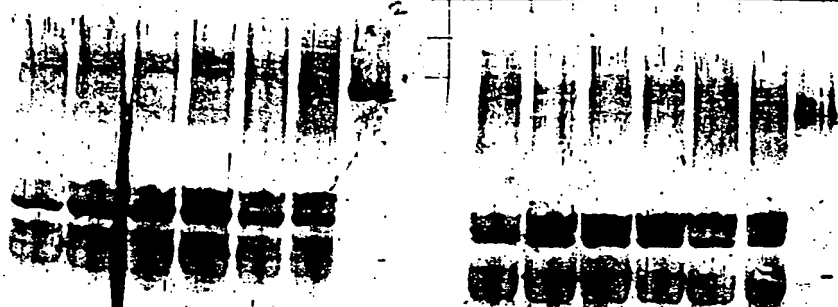
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9th Dec 93

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4th Dec 93

From Page No. _____

9th Dec 93

- Cells in 6 ^{well} ~~plate~~ ^{plates}

Well 1 : FL-16

2 FL-17

3 FL-18

4 FL-19

5 FL-20

6 CHO

Cells were prepared ~20 hrs before by Will Benson

- Inoculated with 250-500 $\times 10^3$ cells; grown o/n in DMEM + serum.

: Microscopic examination suggests about 50% confluency

- Removed media by suction

- washed with 3mls pre-warmed serum free DMEM + HEPES

- Sucked off wash

Added 1ml of serum free DMEM + HEPES.

Incubated at 37°C for 60 mins

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10th Dec 93

K. M. B.

22

Project No. _____
Book No. 19211 TITLE (P)-14K APOL STIMULATION

From Page No. _____

- Added Abs :- 20 μ l of each as shown below
 - This represents a 1/50 dilution of the serum.

PLATE 1 - no additions (unstimulated)

PLATE 2 - 71-B-① 5min stimulation

PLATE 3 71-B-① 30min stimulation

PLATE 4 71-B-② 5min

PLATE 5 71-B-2 30min

PLATE 6 71-B-Preimmune 5 min

PLATE 7 71-B-Preimmune 30mins

① = 10/28/93 bleed

② = 11/18/93 bleed

Preimmune = 8/11/93 bleed

- Removed supernatants after the shown times & washed cells 1x with PBS.

→ Removed PBS

- Added 300 μ l of 1x SB.

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10/11 Dec 93

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TITLE (P-TYR - WESTERNS, AFTER STIMULATION)

Proj. No. _____
Book No. 19211

Exhibit L, pg. 23 of 31

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Transferred to eppendorf tubes. Heated 95°C for 5 min.
Loaded 10^μm (30μl) to 8% PAGE-SDS.

Tubes labelled as follows:

Plate 1 → 1-1 ← Well 1 2-1 3-1 ... etc
 1-2
 1-3
 1-4
 1-5
 1-6

Gels loaded as follows:

GEL 1
Mr FL-16 FL-17
H1 2-1 3-1 4-1, 5-1, 6-1, 7-1 Mr 1-2, 2-2, 3-2, 4-2, 5-2, 6-2, 7-2

GEL 2
Mr FL-18 FL-19
1-3, 2-3, 3-3, 4-3, 5-3, 6-3, 7-3, Mr 1-4, 2-4, 3-4, 4-4, 5-4, 6-4, 7-4

GEL 3
Mr FL-20 CHO
1-5, 2-5, 3-5, 4-5, 5-5, 6-5, 7-5 Mr 1-6, 2-6, 3-6, 4-6, 5-6, 6-6, 7-6

Ran gels slowly o/n.

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10 Dec 93

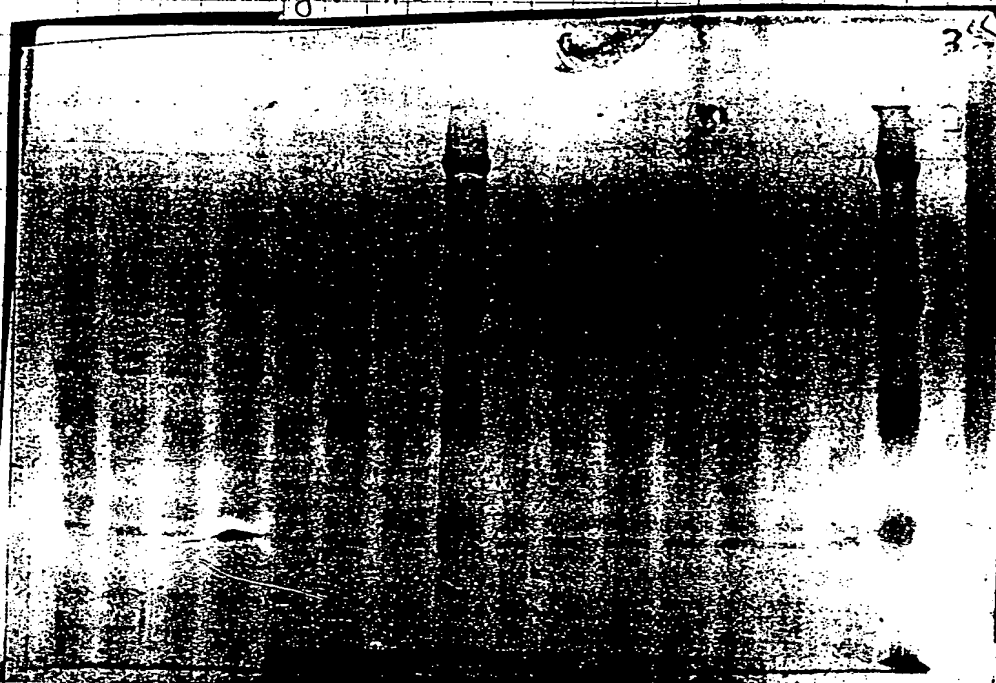
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10th Dec 93

Plotted gels to NYC filter.

Very little protein on the filters; transfer worked
fine because the markers are OK.

- E.g. lane of gel #3.



Decorated gel with UBS's anti- ϕ -tyf monoclonal
Ab.

Blocked filters in PBS / 5% BSA / 0.05% Tween-20.

:- 2-3 hrs room temp.

; wash 3x PBS

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Exhibit L, pg. 25 of 31

From Page No. _____

- 2° Ab was Anti-mouse - Phosphatase (Boehringer)

- 2 hrs in PBS / 5% BSA / Tween-20

- washed 3x PBS

1x Alk Phosph. buffer

{ 100mM Tris-Cl. pH 9.5
100mM NaCl
5mM MgCl₂ }

- Developed with NBT / BCIP.

- No immediate staining was apparent, so
let the staining proceed o/n. Stained gels
attached pages 26+27

RESULTS & CONCLUSIONS

Not enough protein loaded on the gels - need
to repeat the ~~of whole~~ gels + westerns with
these samples.

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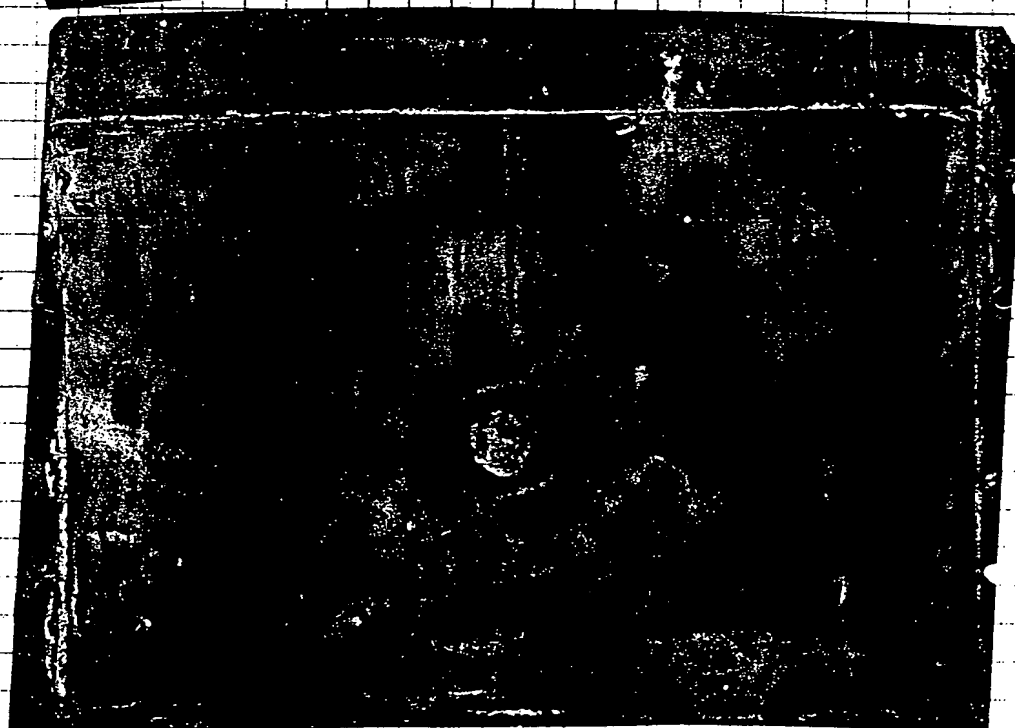
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13th Dec '93

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Book No. 19211 TITLE _____

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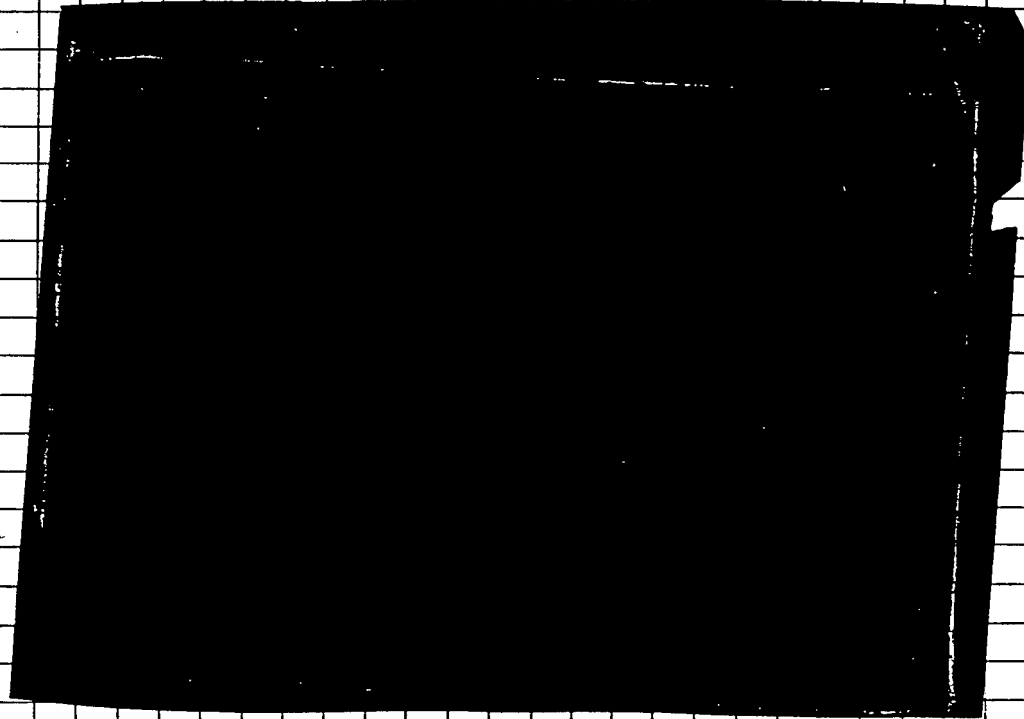
13 Dec 93

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Book No. 19211

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REPEAT OF GELS

13th Dec 93

Loaded 100µl of redwood sample (left top open when
heated to 95°C for 10mins) on to new
8% gels

- Ran gels at 30mA for ~5 hrs

Blotted to N/C filter for ~2 hrs.

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13 Dec 93

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TITLE

(P)-TYR AFTER STIMULATION

Project No.

Book No. 19211

Exhibit L, pg. 29 of 31

From Page No.

14th Dec 93

Decanted the Blots as previously described -
using the MAb (stored at -20°C)

- Developed using NBT/BCIP.

- Blots attached Pages 30 + 31

RESULTS & CONCLUSIONS

No specific bands are obvious after stimulation
with the phorbol & serum; -

There is a prominent band in CHO cells alone, at around
this size which has (P)-Tyr; this may obscure any
bands that are coming up with stimulation.

Alternately there may be no stimulation of kinase
activity with these serum / bleeds; new bleeds should
be tried.

Also keep in mind a metabolic (³⁵S) labelling followed by
anti-(P)-Tyr immuno-ppt.

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15th Dec 93

Project No.
Book No. 19211

31

TITLE _____

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